Short Communication

CYP1A1 I462V Genetic Polymorphism and Lung Cancer Risk in a Cohort of Men in Shanghai, China[1]

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Abstract

Cytochrome P450 (CYP) CYP1A1 activates tobacco-related carcinogens. A point mutation at codon 462 in exon 7 of *CYP1A1* results in a substitution of isoleucine by valine near the heme binding site. This mutation is rare in Caucasians but common in Japanese populations, in which association with increased risk of lung cancer has been reported. There are few data in other Asian populations. We investigated this I462V polymorphism using DNA from 214 incident cases of lung cancer and 669 controls in a prospective cohort study of 18,244 middle-aged and older men in Shanghai, China. The valine allele frequency was 0.138 among the control population. The I462V genotype was not appreciably associated with lung cancer risk overall. There was some suggestion that having at least one valine allele might be related to increased risk of lung cancer among smokers of <20 cigarettes/day (odds ratio, 1.72; 95% confidence interval, 0.82–3.62), particularly among those with homozygous deletion of *GSTM1* (odds ratio, 2.80; 95% confidence interval, 1.07–7.33), which is involved in the detoxification of activated tobacco carcinogens. In this Chinese cohort, with *CYP1A1* valine allele frequency intermediate between Japanese and Caucasian populations, the I462V polymorphism is not related to lung cancer overall, but it might play a role at lower levels of cigarette smoking among subjects with impaired carcinogen detoxification as assessed by the *GSTM1*-null genotype.

Introduction

The CYP[3] CYP1A1 is involved in the metabolic activation of benzo(*a*)pyrene, a major carcinogen in tobacco smoke, and is expressed in the human lung (1). A genetic polymorphism resulting in an amino acid change from isoleucine to valine at codon 462 in the heme-binding region was first described in 1991 by Hayashi *et al.* (2). This polymorphism has been shown to correlate with inducibility of aryl hydrocarbon hydrolase activity (3).

In Japan, where the valine allele is relatively common (frequency, 0.20–0.25), the valine allele has been associated with increased risk of lung cancer (4–6). In some studies, this association is stronger among subjects with a homozygous deletion of the *GSTM1* gene (6–8), which is involved in the detoxification of benzo(*a*)pyrene-7,8-diol-9,10-oxide, the ultimate carcinogen metabolite of benzo(*a*)pyrene (9). Reports from Caucasian and African-American populations with lower allele frequencies have been less consistent [as reviewed recently by Bartsch *et al.* (10)]. Although the frequency of the valine variant allele is higher in Chinese populations (11, 12) than in Caucasian populations, there are few data on Chinese populations.

We therefore examined the relation between this *CYP1A1* polymorphism and lung cancer risk among incident cases of lung cancer and controls from a cohort study of men in Shanghai, China using DNA extracted from serum. We also considered possible effect modification by null genotypes of *GSTM1* and *GSTT1* and cigarette smoking.

Materials and Methods

Study Population. Subjects were drawn from a prospective study of men in Shanghai, China. Details of the cohort have been published previously (13, 14). In brief, between January 1, 1986 and September 30, 1989, all male residents of four small geographically defined communities in Shanghai who were age 45–64 years and had no history of cancer were invited to participate in an epidemiological study. At enrollment, subjects provided a 10-ml blood sample and a single void urine. Samples were stored at −20°C. Each subject was interviewed in person using a structured questionnaire that included demographic information, history of tobacco and alcohol use, current diet (45 items), and medical history. A total of 18,244 men were enrolled, constituting 80% of eligible subjects. Follow-up has been conducted by annual contacts with all surviving cohort members and a twice yearly review of cancer reports from the Shanghai Cancer Registry and of death certificates. To date, only 120 subjects have become lost to follow-up.

Through follow-up ending March 15, 1997, we identified 259 incident cases of lung cancer. Of the 259 cases, 178 cases were confirmed histopathologically, and 81 cases were based on clinical diagnosis including radiography or computer-assisted tomography. For each case of incident lung cancer, three controls were drawn with matching on neighborhood of residence, age at interview (within 2 years), and month of sample collection. For all laboratory assays, the matched sets were analyzed together in a blinded manner.

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[3] The abbreviations used are: CYP, cytochrome P450; GST, glutathione *S*-transferase; OR, odds ratio; CI, confidence interval.

Laboratory Methods. DNA was extracted from serum according to the following procedure: 1.5 ml of serum were spun at 14,000 × *g* for 5 min, and the pellet was dissolved in 20 μl of 0.05 N NaOH and heated to 98°C for 10 min. Then, 2.5 μl of 1 M Tris (pH 8) were added to neutralize the NaOH, and the solutions were stored at −20°C until PCR analysis. One μl of this crude DNA preparation was usually used per PCR reaction. DNA extracted from spun serum has been shown to be a reliable and representative source of constitutional DNA for genotyping of polymorphisms including *CYP1A1 I462V* and *GSTM1* (15).

A PCR-based RFLP assay for the I462V polymorphism of *CYP1A1* was used (16). Briefly, this method used the following two primers: (*a*) *CYP1A1*F (forward), 5′-GAAAGGCTGGGTCCACCCTCT; and (*b*) *CYP1A1*R (reverse), 5′-CCAGGAAGAGAAAGACCTCCCAGCGGGCCA. Due to the small amounts of DNA in our serum samples, we used a double PCR strategy with sequential PCR reactions using the same published conditions and primers. Ten μl of the first PCR reaction were used in a secondary PCR reaction that contained 2 μCi of [α-^{32}P]dCTP in addition to the other components. Ten μl of this reaction were then subjected to restriction enzyme analysis with *Nco*I (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. Genotyping was then scored after resolution on 5% acrylamide sequencing gels containing 7 M urea, followed by exposure to photographic film (Kodak XAR5). The wild-type allele (Ile) was distinguished by the presence of a 232-bp fragment, whereas a 263-bp fragment identified the variant allele (Val).

Heterozygous samples (Ile/Val) showed two bands: (*a*) one of 263 bp; and (*b*) one of 232 bp. The DNA samples were adequate for determination of *CYP1A1* genotype for 214 cases and 669 controls.

A multiplex PCR protocol was used to simultaneously analyze samples for the presence or absence of *GSTM1* and *GSTT1* genes as described by Arand *et al.* (17), with the following modifications. All primers (*GSTM1*, *GSTT1*, and albumin) were at final concentrations of 50 pmol/30 μl PCR reaction. The polymerase was rTaq (Pharmacia, Piscataway, NJ), and 5% DMSO was included. The annealing temperature was 55°C, and 36 cycles were used. This reaction was followed by two independent secondary PCR reactions, each containing primers for *GSTM1* and *GSTT1*, respectively, and in each case, 10 μl of the first PCR reaction were used as a template. Each secondary reaction also contained 2 μCi of [α-^{32}P]dCTP to allow for autoradiographic detection of products that were resolved on 5% acrylamide sequencing gels containing 7 M urea. The dried gels were exposed to photographic film (Kodak XAR5) for 2–6 days. The presence or absence of the particular PCR product was scored, and subjects were classified according to whether or not they had a homozygous deletion of each gene. No attempt was made to distinguish null/+ heterozygotes from +/+ homozygotes. For samples scored as having a homozygous deletion for both *GSTM1* and *GSTT1*, a third secondary PCR reaction using only primers for albumin was performed and analyzed as described above. If an albumin PCR product could be identified, subjects were finally scored as having a homozygous deletion for both *GSTM1* and *GSTT1*. We scored all samples where none of the three products could be identified as noninformative. Among subjects with *CYP1A1* data, we were able to determine *GSTM1* and *GSTT1* genotypes of 202 cases and 628 controls.

Statistical Analysis. Data were analyzed using SAS (Cary, NC) software version 6.12. For analyses involving all subjects, we compared relative risks (estimated from ORs) based on conditional logistic regression methods (Proc Phreg) with relative risks based on unconditional logistic regression methods (Proc Logistic). In unconditional logistic regression runs, age (as a continuous term) was included as a covariate. The addition of terms for neighborhood, month and year of enrollment (matching factors), and education (as a surrogate for neighborhood social factors) did not appreciably alter the unconditional results and thus were not retained. Conditional logistic regression analysis gave ORs of 0.91 (95% CI, 0.60–1.37) for the Ile/Val genotype and 1.09 (95% CI, 0.48–2.48) for the Val/Val genotype relative to the Ile/Ile genotype. Corresponding ORs from unconditional logistic regression were 0.93 (95% CI, 0.62–1.38) and 0.91 (95% CI, 0.41–2.05). Because results were comparable in matched and unmatched analyses, we present the results from unconditional logistic regression runs to maximize the number of subjects included in the various analyses, particularly for stratified analyses. We adjusted for smoking by including terms for smoking status (never, current, or past smoker), age at starting to smoke, and the average number of cigarettes smoked per day. As seen in Table 1, adjustment for smoking did not substantially alter the age-adjusted associations, and only smoking-adjusted ORs are presented in Table 2. These terms have previously been determined to best capture the smoking-lung cancer association in this cohort (14).

Results

The mean age at interview was the same for cases and controls (58 years; SD, 5 years), consistent with the age matching. The mean age at lung cancer diagnosis was 63 years (SD, 6 years). At the time of the initial interview, 47.5% of controls and 82.2% of cases reported being current smokers. The high rates of smoking in this cohort are representative of middle-aged men in China during this time period (14). Past smoking was uncommon and equally distributed between cases and controls (7.9%). Among smokers, the mean number of cigarettes smoked per day was 21 (SD, 8 cigarettes/day) for cases and 15 (SD, 8 cigarettes/day) for controls.

Among all 669 controls, the frequency of the valine allele was 0.138 (95% CI, 0.119–0.156). Among controls, the presence of the valine allele was not related to smoking behavior or age. The frequency of the valine allele among our cases was 0.129 (95% CI, 0.096–0.162).

The I462V polymorphism was not related to lung cancer risk in subjects overall (Table 1). Histologically confirmed cases (n = 152) were similar to those without histological confirmation (n = 62) based on age at diagnosis, smoking status, and education (data not shown). There was no association between CYP1A1 and lung cancer overall within either of these two subgroups (data not shown). When we stratified by cell type, risk estimates become rather unstable because of the small sample sizes. Nevertheless, we did not find associations within the group with squamous cell or small cell carcinoma (74 cases), within the group with adenocarcinoma (64 cases), or within the group with other and unknown cell types (76 cases; Table 1).

Results were not appreciably altered when analyses were restricted to smokers. This is as expected, given the lack of appreciable association between smoking and *CYP1A1* genotype and the small number of nonsmoking cases (Table 2). Among subjects who smoked less than the median amount of 20 cigarettes/day, there was a nonstatistically significant elevation in the risk of lung cancer for carrying at least one valine allele (OR, 1.72; 95% CI, 0.82–3.62). Stratification by total

Table 1 *CYP1A1* I462V polymorphism in relation to lung cancer among a cohort of older middle aged men in Shanghai, China

CYP1A1 genotype	Cases		Controls		Age-adjusted OR[b]	Age- and smoking-adjusted OR (95% CI)[c]
	n	%[a]	*n*	%		
All subjects	214		669			
Ile/Ile	167	78.0	512	76.5	1.00	1.00
Ile/Val	39	18.2	130	19.4	0.93	0.95 (0.62–1.48)
Val/Val	8	3.7	27	4.0	0.91	0.85 (0.33–2.20)
Ile/Val or Val/Val	47	22.0	157	23.5	0.93	0.94 (0.62–1.41)
Histology[d]						
Adenocarcinoma	64					
Ile/Ile	53	82.8				
Ile/Val	11	17.2			0.80	0.83 (0.41–1.67)
Val/Val	0	0				
Ile/Val or Val/Val	11	17.2			0.66	0.70 (0.35–1.41)
Squamous & small cell	74					
Ile/Ile	54	73.0				
Ile/Val	18	24.3			1.33	1.38 (0.74–2.55)
Val/Val	2	2.7			0.71	0.58 (0.08–4.04)
Ile/Val or Val/Val	20	27.0			1.22	1.27 (0.70–2.31)
Other and unknown	76					
Ile/Ile	60	78.9				
Ile/Val	10	13.2			0.68	0.69 (0.33–1.45)
Val/Val	6	7.9			1.91	1.49 (0.50–4.48)
Ile/Val or Val/Val	16	21.1			0.89	0.84 (0.44–1.59)

[a] Totals may add to slightly more or less than 100% due to rounding.
[b] OR, calculated relative to Ile/Ile homozygotes. Age was modeled as a continuous variable.
[c] 95% CI. Smoking-adjusted model includes terms for number of cigarettes/day, age started smoking, and smoking status (never, past, or current).
[d] The comparison group for each histology category is all controls combined.

lifetime cigarette consumption (years smoked times the number of cigarettes/day) divided at the 60th percentile of the overall distribution to give stable numbers did not produce any appreciable difference in risk for the two categories of smokers (data not shown). However, in this cohort, the number of cigarettes smoked per day was more strongly related to lung cancer risk than cumulative smoking (14).

GSTM1 is involved in detoxification of the activated metabolites of tobacco carcinogens, including benzo(*a*)pyrene, produced by the action of CYP1A1 (9). Previous data from Japan suggest that the valine allele is most strongly associated with lung cancer risk when the *GSTM1*-null genotype is present (6–8, 18). In our study, among smokers with the *GSTM1*-null genotype, those with at least one valine allele were at slightly increased risk of lung cancer, but the association was not statistically significant (OR, 1.52; 95% CI, 0.86–2.71). The number of subjects with the Val/Val genotype is small, and no increased risk was observed in this category. When we considered *GSTM1* genotype and the number of cigarettes/day simultaneously, an increased risk of lung cancer in relation to carrying a valine allele was most notable among lighter smokers who were also *GSTM1* null (OR, 2.80; 95% CI, 1.07–7.33). The association between the I462V polymorphisms and lung cancer did not vary appreciably by *GSTT1* genotype (data not shown).

We have reported recently in this cohort that detectable levels of isothiocyanates in the urine are associated with reduced risk of lung cancer, particularly among *GSTM1*-null individuals (19). Although estimates become unstable on further stratification, we did not find evidence that the urinary levels of isothiocyanates modify the relation between the *CYP1A1* I462V polymorphism and lung cancer risk either overall or according to *GSTM1* genotype (data not shown).

Discussion

The *CYP1A1* I462V polymorphism was not significantly associated with lung cancer risk overall or with individual cell types, in contrast to the findings of some previous studies in Japan (4, 6). Consistent with previous studies in Japanese populations, the presence of a valine allele was associated with a modest but nonstatistically significant increase in lung cancer risk among smokers with lower daily cigarette consumption (4, 18) or among those with the *GSTM1*-null genotype (6–8). The presence of a valine allele was most strongly associated with lung cancer risk among smokers with both the *GSTM1*-null genotype and lower daily cigarette consumption, a finding consistent with data from Japan (18). Caution is required in interpreting the finding of increased risk for carriers of the valine allele with lower cigarette consumption combined with the *GSTM1*-null genotype because the numbers become small on cross-classification. However, it is biologically plausible that at lower levels of tobacco smoke exposure, the relatively minor variability in carcinogen metabolism due to genetic polymorphisms may be more important than at higher exposure levels, where numerous direct effects of smoking predominate.

An association between the I462V polymorphism and lung cancer has been most consistently seen in studies in Japan (4–6), where the frequency of the valine allele is substantially higher than that in the Caucasian or African-American populations. The association between the I462V polymorphism and lung cancer overall has been less consistently reported in Caucasian and/or African-American populations (10). The frequency of the valine allele in Chinese is intermediate between the frequencies in Japanese and non-Asian populations. In the English literature, we have found only one other study of the I462V polymorphism in relation to lung cancer from a Chinese population (12). In that study of only 76 cases, no statistically significant associations were seen (12).

The allele frequency in our controls (0.138; 95% CI, 0.119–0.156) was consistent with that of three smaller control series from other areas of the People's Republic of China: (*a*) 0.141 (95% CI, 0.057- 0.225; Ref. 4), (*b*) 0.197 (95% CI, 0.144–0.250; Ref. 12); and (*c*) 0.207 (95% CI, 0.149–0.267;

Table 2 *CYP1A1* I462V polymorphism in relation to lung cancer by smoking history and *GSTM1* genotype among smokers

Category	Cases		Controls		OR (95% CI)[b]
	n	%[a]	*n*	%	
All smokers	184		348		
Ile/Ile	140	76.1	273	78.4	1.00
Ile/Val	37	20.1	66	19.0	1.09 (0.67–1.75)
Val/Val	7	3.8	9	2.6	0.98 (0.33–2.94)
Ile/Val or Val/Val	44	23.9	75	21.6	1.07 (0.68–1.68)
<20 cigarettes/day	49		188		
Ile/Ile	35	71.4	151	80.3	1.00
Ile/Val	14	28.6	35	18.6	1.78 (0.85–3.75)
Val/Val	0	0.0	2	1.1	
Ile/Val or Val/Val	14	28.6	37	19.7	1.72 (0.82–3.62)
20+ cigarettes/day	135		160		
Ile/Ile	105	77.8	122	76.3	1.00
Ile/Val	23	17.0	31	19.4	0.84 (0.45–1.54)
Val/Val	7	5.2	7	4.4	0.99 (0.32–3.06)
Ile/Val or Val/Val	30	22.2	38	23.8	0.86 (0.49–1.51)
GSTM1 null	98		208		
Ile/Ile	68	69.4	164	78.8	1.00
Ile/Val	27	27.6	37	17.8	1.69 (0.92–3.11)
Val/Val	3	3.1	7	3.4	0.78 (0.19–3.26)
Ile/Val or Val/Val	30	30.6	44	21.2	1.52 (0.86–2.71)
GSTM1 positive	86		140		
Ile/Ile	72	83.7	109	77.9	1.00
Ile/Val	10	11.6	29	20.7	0.52 (0.23–1.16)
Val/Val	4	4.7	2	1.4	1.72 (0.23–12.9)
Ile/Val or Val/Val	14	16.3	31	22.1	0.60 (0.28–1.27)
GSTM1 null, <20 cigarettes/day	31		115		
Ile/Ile	20	64.5	94	81.7	1.00
Ile/Val or Val/Val	11	35.5	21	18.3	2.80 (1.07–7.33)
GSTM1 null, 20+ cigarettes/day	67		93		
Ile/Ile	48	71.6	70	75.3	1.00
Ile/Val or Val/Val	19	28.4	23	24.7	1.13 (0.55–2.34)
GSTM1 positive, <20 cigarettes/day	18		73		
Ile/Ile	15	83.3	57	78.1	1.00
Ile/Val or Val/Val	3	16.7	16	21.9	0.66 (0.17–2.65)
GSTM1 positive, 20+ cigarettes/day	68		67		
Ile/Ile	57	83.8	52	77.6	1.00
Ile/Val or Val/Val	11	16.2	15	22.4	0.62 (0.25–1.55)

[a] Total may add up to less than 100% due to rounding. Smokers with missing data on *GSTM1* genotype are excluded form analyses for this table (9 cases and 23 controls).
[b] ORs and 95% CIs are calculated relative to Ile/Ile homozygotes. Models include terms for smoking (past *versus* current) and continuous terms for age, number of cigarettes smoked per day, and age at starting to smoke.

Ref. 11). We observed a deviation from Hardy Weinberg equilibrium that was similar in cases and controls: a deficit of Ile/Val and an excess of Val/Val genotypes. Given the large size of our control group (the largest study of CYP1A1 and any cancer included in a recent comprehensive review; Ref. 10), this deviation was statistically significant. In most previous studies, the ability to detect a deviation from Hardy Weinberg equilibrium would be low because the test for deviation has low power in small samples (20) and even more so when the allele is rare, as in studies of Caucasians. We repeated all genotypes to rule out laboratory error. We are thus left with the usual explanations for deviation from Hardy Weinberg equilibrium: (*a*) drift; (*b*) population admixture; (*c*) nonrandom mating; and (*d*) selection (20). Although there are few data on Chinese populations, in two studies, the same pattern that we observed (a deficit of Ile/Val and an excess of Val/Val) was seen (11, 21). Were these same proportions were to hold if these two control groups were as large as ours, these deviations would be statistically significant. In a study in a Korean population, Hardy Weinberg equilibrium was not observed (22).

In this study, the same pattern of deviation from Hardy Weinberg equilibrium was observed for cases and controls. Thus, it is unlikely that control selection bias occurred based on genetic factors. Hardy Weinberg equilibrium, while required for tests based on alleles, is not required for the calculation of ORs by genotype, the standard epidemiological analysis (23). An advantage of our nested case-control study design is that the controls were sampled from the population at risk and are thus representative of the population that gave rise to the cases. This reduces the possibility of selection bias compared with case-control studies, where hospital controls or convenience samples were used because of the difficulty of achieving high response rates among general population controls when phlebotomy is required.

We measured the *CYP1A1* I462V polymorphism but not the *Msp*I polymorphism. The *Msp*I polymorphism, which has a higher variant allele frequency, has been reported to be more strongly associated with lung cancer risk than the I462V polymorphism in some studies, and significant associations have sometimes been found only when the two polymorphisms were considered simultaneously (10). Given the challenges of genotyping the small quantity of DNA found in serum, we focused on the I462V polymorphism because it results in a nonconservative amino acid change in the heme-binding region of the

protein, whereas the *Msp*I polymorphism occurs in an intron. The I462V polymorphism has been shown to correlate with aspects of enzyme function including constitutive aryl hydrocarbon hydroxylase activity (3), CYP1A1 enzyme activity, and *CYP1A1* mRNA induction (24). However, neither the I462V nor the *Msp*I polymorphism has been shown to actually alter the metabolic activation of benzo(*a*)yrene or other tobacco-related carcinogens.

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